



GEIS Respiratory Disease Surveillance Newsletter

DoD Center for Deployment Health Research
Naval Health Research Center, San Diego



Issue 7

Spring 2004

The DoD Center for Deployment Health Research at the Naval Health Research Center (NHRC) serves as the Navy hub for the DoD Global Emerging Infections System (GEIS). Through this system, NHRC collaborates with numerous institutions to conduct surveillance for respiratory pathogens, including adenovirus, influenza, respiratory syncytial virus, parainfluenza, *Bordetella pertussis*, *Streptococcus pyogenes*, and invasive *Streptococcus pneumoniae*. For more detailed information about NHRC DoD-GEIS projects, please visit our website at <http://www.nhrc.navy.mil/geis>.

SARS Testing at NHRC

In June 2003, NHRC was designated as a Laboratory Response Network (LRN) site for Severe Acute Respiratory Syndrome (SARS). Facilities with suspected cases of SARS can send specimens to NHRC for screening, where they will be tested using reagents and protocols provided by CDC. SARS-positive cases will be reported through the appropriate channels and specimens forwarded to CDC for confirmation.

2003-04 Influenza Update

To date, we have confirmed 126 influenza A (H3N2) cases among basic trainees during this season. Influenza has been seen at all 8 training centers participating in febrile respiratory illness (FRI) surveillance. A subset of isolates has been sequenced, and all show the amino acid substitutions characteristic of the A/Fujian/411/2002 strain. Only 14/126 (11.1%) cases occurred among trainees vaccinated ≥ 14 days. Using this population and data, estimates of vaccine efficacy are being pursued. No influenza B has been seen thus far this season.

FRI Rates Categorized Using ARIMA Modeling

Since the mid-1980's, a single, fixed threshold for FRI epidemics (1.5 cases/100 trainees/week) has been in use. This static threshold was adopted during use of the oral adenovirus vaccine, and may be less appropriate now that the vaccine is not available. To this end, we recently began using autoregressive integrated moving average (ARIMA) modeling in conjunction with fixed thresholds to categorize rates on a site-by-site basis. Each week at each site, FRI Rate Status is classified as either at/below expected, moderately elevated, or substantially elevated. More information about FRI Rate Status can be found on our web site, www.nhrc.navy.mil/geis. If you would like to receive a weekly email update of FRI rate status, please contact us at FRI@nhrc.navy.mil.

Current Study Updates

Laboratory Based Surveillance for Febrile Respiratory Illness Aboard Floating Platforms

FRI surveillance studies were expanded in late 2002 to include Navy ships in the Pacific Fleet. These ships travel to areas that have been associated with emerging viral pathogens such as SARS and new strains of influenza. Surveillance is currently ongoing aboard 7 ships, and 2 more will be added soon. We have received more than 130 specimens to date, and found mild outbreaks of Fujian-like influenza A (H3N2) among sailors aboard 2 ships that occurred shortly after port stops. One ship had stopped in Pearl Harbor, HI (March 2003), and the other had stopped in Sydney, Australia (July 2003). Three influenza B isolates have also been identified.

Real-time Diagnostic Capabilities on Aircraft Carriers – Real-time PCR techniques for influenza and adenovirus detection have been deployed on a few floating platforms. Routine use of this equipment, while testing for respiratory pathogens, will help to maintain technical proficiency when needed to rule-out bioterrorism agents.

Evaluation of PCR Testing Using Room Temperature Specimens

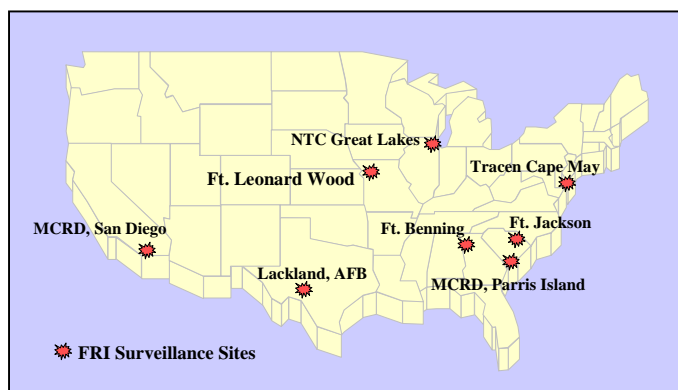
One of the biggest challenges FRI surveillance sites face is keeping viral specimens frozen at ultra-low temperatures for long periods before and during shipping. A sub-study at Fort Jackson was conducted to evaluate the performance of room temperature specimens in comparison with frozen viral culture. The study, in collaboration with researchers at the Armed Forces Institute of Pathology, used PCR techniques to test for adenovirus and influenza among specimens that have been stored at room temperature.

Specimens collected during the 2002-2003 influenza season showed good sensitivity and specificity for influenza A (75% and 98%) and influenza B (86% and 99%). A similar study is being conducted during the 2003-04 influenza season to increase sample size and adapt PCR methods at NHRC.

Association Between Adenovirus Infections Among Military Personnel and the Development of Obesity

This case control study, which began in October 2001, investigates if an association exists between weight control problems (obesity) and adenovirus-36 exposure in a population of active-duty Navy personnel. The study, which is being conducted at the Clinical Trials Center and at other military commands in San Diego, is currently enrolling cases (individuals with a Body Mass Index (BMI) of >30) and controls (BMI <25).

Febrile Respiratory Illness Surveillance



Current Progress – Febrile Respiratory Illness surveillance continues at eight military training sites. Specimens are collected from trainees presenting with symptoms meeting the FRI case definition (oral temperature $\geq 100.5^{\circ}\text{C}$ and cough or sore throat, or any case of radiographically confirmed pneumonia). Specimens are tested for adenovirus, influenza A and B, respiratory syncytial virus (RSV), and parainfluenza 1, 2 and 3. The specific trends observed for each of these viruses are described in the following sections.

Influenza

Current Progress – During the 2003-04 season, 126 influenza A (H3N2) cases have been confirmed to date among the recruit training population, and 16% of all specimens were positive for influenza A during this period. More information about the current season can be found on page 1. Since FRI surveillance began in 1998, 457 of 13,282 (3.4%) specimens have been positive for influenza, with 73% of those being influenza A and 27% influenza B. During the 1998-2003 time frame, ill trainees who were not vaccinated against influenza were almost 5 times more likely to be influenza-positive (OR= 4.8, 95% CI 3.8-6.0) than those who did receive the vaccine.

Adenovirus

Current Progress – In the absence of vaccine, adenovirus remains the leading cause of FRI among trainees. Sixty-five

percent of the 13,282 throat cultures collected for the FRI study between June 1998 and September 2003, and 2,144 (75.4%) of the 2,845 collected between October 2002 and September 2003, tested positive for adenovirus. Approximately 98% of all adenovirus isolates collected to date have been type 4.

Other FRI Study Pathogens

Of the 13,282 throat cultures tested thus far under the FRI study, 21 (0.2%) have been positive for RSV and 86 (0.9%) have been positive for parainfluenza 1, 2, or 3. Of the 2,845 specimens collected from October 2002 to September 2003, 23 (0.8%) have been positive for parainfluenza 1, 2, or 3; none of these samples were positive for RSV.

***Streptococcus pyogenes* Surveillance**



Current Progress – As demonstrated by a GAS pneumonia outbreak at MCRD San Diego in December 2002, *Streptococcus pyogenes* (Group A streptococcus [GAS]) continues to be a threat to the health of military trainees. GAS-positive clinical isolates continue to be collected from laboratories at 8 military training sites across the nation. One thousand six hundred and eighteen have been collected thus far, 604 in the last year. Antibiotic resistance testing was performed on all isolates, and emm-gene sequencing was performed on a set of randomly selected samples.

Antibiotic Resistance – Among the specimens tested, *S. pyogenes* maintains 100% susceptibility to the antibiotics penicillin and vancomycin. Two hundred and fourteen (13.2%) of the 1,618 isolates collected demonstrated full or partial resistance to erythromycin, 78 (4.8%) to tetracycline, 35 (2.2%) to clindamycin, and 19 (1.2%) to levofloxacin. Twenty-seven (1.7%) of the isolates were resistant to both erythromycin and tetracycline.

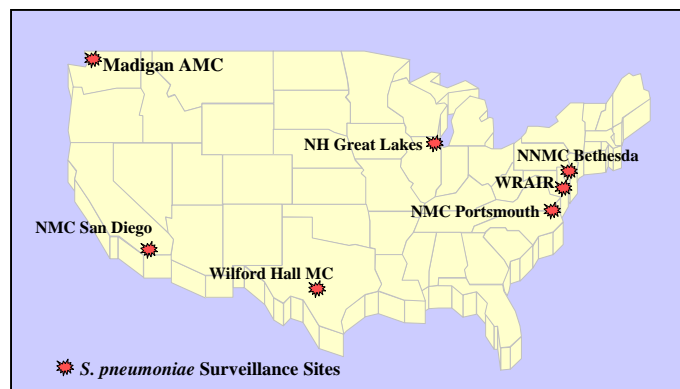
Emm-gene Types – As of September 2003, emm-gene sequencing had been performed on 825 randomly selected samples. The most common emm types were 3 (20%), 29 (15%), 6 (12%), 44/61 (8%), 2 (6%), 1 (6%), 75 (6%), and 12 (4%). These eight emm-gene types made up more than 77% of all typed isolates.

Continued from page 2

Resistance by Emm-gene type – Erythromycin resistance varied by emm-gene type, with Type 75 demonstrating by far the most resistance of all emm-gene types (61% fully resistant). Most (67%) Type 75 isolates were from trainees at Lackland AFB, TX. A manuscript of this work has just been published; Barrozo CP, et. al. National Department of Defense surveillance data for antibiotic resistance and emm gene type of group A streptococcal isolates from eight basic training military sites. *J Clin. Microbiology* 2003; 41(10):4808-11.

Geographic Trends – *S. pyogenes* isolates from military trainees continue to maintain susceptibility to many commonly prescribed antibiotics. However, we continue to observe an unequal geographic distribution of erythromycin resistance at the sites. Resistance to erythromycin differed between sites, with Fort Leonard Wood having the highest resistance rate (34.9%). Ninety-nine percent of the erythromycin resistant isolates from Fort Leonard Wood were fully resistant.

Streptococcus pneumoniae Surveillance



Current Progress – Invasive clinical isolates continue to be collected from 7 military medical centers across the nation. Three hundred and eighty-three isolates have been collected thus far, 15 within the last year.

Antibiotic Resistance – One hundred and thirty (33.9%) of the 383 isolates collected to date demonstrated full or partial resistance to penicillin, and 85 isolates (22.2%) exhibited multiple resistance to three or more antibiotics. Penicillin resistance among males (35.0% resistant) and females (32.5% resistant) was similar.

Resistance by Serotype – Penicillin resistance differed by serotype, with types 19 (54%), 9 (50%), and 6 (48%) demonstrating the most resistance. These three serotypes accounted for more than 62% of all penicillin resistance among the serotyped isolates, though they only comprised 43% of the total number of serotyped isolates.

Bordetella pertussis Surveillance

Surveillance of *Bordetella pertussis* has ceased at Fort Benning and Fort Leonard Wood, and continues at MCRD

San Diego and NRTC Great Lakes. Site personnel have enrolled 292 subjects to date. Of these, 147 specimen sets have been tested using 3 different methods. These specimens were collected from recruits meeting the case definition for pertussis, which is more than seven days of coughing with symptoms consistent with a respiratory infection. Preliminary results show that at least one (0.6%) of the specimens tested positive by culture, 9 (6.1%) were positive by PCR, and 3 (2%) tested positive by serology (seroconversion). To date, all positives have been from MCRD San Diego.

Expanding Projects in Molecular Epidemiology

Sequencing of Adenovirus Isolates from Previously Vaccinated Individuals

Through respiratory surveillance, NHRC has discovered that from 1996 to 1999 several individuals who received the adenovirus vaccine subsequently became ill with adenovirus 4 or 7 infection. It is important to understand why these vaccinated individuals became ill. Was it simply a poor immune response, or was there molecular evolution occurring in the virus that allowed it to “break through” the vaccine? Research by Drs. Dean Erdmann, Lita Crawford-Miksza, and David Schnurr has shown significant variation in the epitope-coding portions of the hexon gene. NHRC, in collaboration with Luke Daum at Brooks Air Force Base, has sequenced and phylogenetically analyzed the hexon gene of each breakthrough strain to determine if genetic drift contributed to these break-through infections. A manuscript of this work has been accepted for publication in the April 2004 issue of the *Journal of Clinical Microbiology*.

Multiplex-PCR Subtyping of Adenovirus

NHRC has developed a multiplex-PCR assay to replace microneutralization as its current adenovirus typing method. This technique is currently undergoing validation testing. The benefits of PCR testing include cost-effectiveness and the elimination of dependence on type-specific antisera, which is not commercially available.

Retrospective Molecular Surveillance for Human Metapneumovirus, Rhinovirus, and Coronaviruses OC43 and 229E

A new respiratory virus, human metapneumovirus (hMPV), was discovered in June 2001. The clinical symptoms of hMPV are similar to those of human respiratory syncytial virus and include mild respiratory problems, cough, bronchiolitis, pneumonia, high fever, myalgia, and vomiting. The epidemiology of this pathogen needs further elucidation in our population, including prevalence as a co-infection. NHRC has found hMPV among FRI specimens that were negative for our standard respiratory panel. Intensive testing of banked specimens is underway, which will allow us to estimate the burden of hMPV in the recruit population. A manuscript is currently being developed.

Triangulation Identification for Genetic Evaluation of Risks

Ibis Therapeutics is developing a high-throughput, single-pass process for the simultaneous detection of a large number of infective threat agents, named Triangulation Identification for Genetic Evaluation of Risks (TIGER). The process uses PCR primers to amplify housekeeping genes that are conserved across species. The PCR product is run through a highly sensitive mass spectrometer, and each pathogen can be identified by a unique pattern. TIGER has recently been successful in characterizing the serotype of adenovirus. The TIGER technology was also successfully implemented on a selection of GAS isolates from the recent outbreak at the Marine Corps Recruit Depot in San Diego. Additional respiratory pathogens will be utilized to further develop this methodology.

Upcoming Protocols

Correlation Between Environmental Sampling and Adenovirus Illness Among Military Recruits

This study aims to assess the temporal relationship between adenovirus-positive environmental and air samples and adenovirus outbreaks among recruits. By characterizing transmission dynamics more effectively, the results of this study can help to determine more effective mechanisms to predict increased illness, prevent transmission, and ultimately decrease morbidity. The TIGER technology discussed above is being utilized and evaluated in this study.

Evaluation of the Effects of Multiple Vaccinations Administered in a Stressful Environment on Immunologic Function

Chronic, unexplained, multi-symptom illnesses are an increasing challenge in military and veteran populations. Some studies suggest that multiple vaccinations in a stressful environment could lead to chronic multi-symptom illness. The objectives of this study are to determine whether multiple simultaneous vaccinations given in a stressful environment 1) induce a potentially harmful immune response (Th2 shift) and/or 2) are causative of adverse health effects. This study will provide much needed information on the effects of multiple vaccinations, and potentially affect how vaccines are administered throughout the DoD.

Etiology and Epidemiology of Pneumonia Among High Risk Military Trainees

Surveillance of pneumonia is being established at 4 recruit training centers in order to determine incidence and etiology of pneumonia in the absence of pneumococcal vaccination. This will help to establish baseline pneumonia data in this population.

Multiplex PCR for Atypical Pneumonia

Between 15 and 50% of atypical pneumonia is caused by four bacterial pathogens: *L. pneumophila*, *C. pneumoniae*, *M. pneumoniae* and *B. pertussis*. Individual PCR tests exist for each of these pathogens, but their use is costly and time-consuming. We are developing a multiplex PCR test that will test for all 4 pathogens simultaneously, reducing analysis time and materials cost by 75%.

National Surveillance for Adenovirus

NHRC will participate in a national adenovirus surveillance program that will be based at the University of Iowa. The project will examine human and viral risk factors for severe adenovirus infections in the US. NHRC will contribute to the project a sample of adenovirus isolates found among recruits.

Molecular Epidemiology of Adenovirus Type 4 Respiratory Infections in Military Populations

We are collaborating with the Lovelace Institute in New Mexico in examining the molecular epidemiology of adenovirus type 4 infections. DNA-level changes that have occurred over time may be important as the new adenovirus vaccine is being prepared for manufacture.

Completed Studies

Pneumococcal Vaccine Trial

In collaboration with Centers for Disease Control and Prevention, the Mayo Clinic and Foundation, and Wyeth Pharmaceuticals, NHRC and 4 recruit training sites are conducting a double blind, placebo-controlled trial of a 23-valent pneumococcal vaccine to assess the vaccine's clinical effectiveness among the military trainee population. Enrollment and surveillance is complete at all sites. The data is currently being processed and the team estimates analysis will begin in the spring. Study site personnel enrolled more than 152,000 recruits with an enrollment rate of approximately 75 percent.

New Personnel

Welcome New Study Personnel

- ◆ **Kevin Gratwick** – Molecular Technician, NHRC
- ◆ **Angel Osuna** – Molecular Technician, NHRC
- ◆ **Charmaine Lawery** – FRI Surveillance Team, Fort Benning

Presentations and Posters

Presentations & Posters at Recent Conferences

International Conference on Emerging Infectious Diseases, Feb 29-Mar 03, 2004, Atlanta, GA.

- ◆ Strickler JK, et al. Symptom and demographic associations with adenovirus infection among U.S. military trainees with febrile respiratory illness.
- ◆ Russell KL, et al. Human metapneumovirus infection among U.S. military recruits: prevalence and molecular analysis.

The Forty-First Annual Meeting of the Infectious Disease Society of America, October 9-12, 2003, San Diego, CA.

- ◆ Blasiole D, et al. Genetic analysis of adenovirus isolates from previously vaccinated military personnel.
- ◆ Kammerer P, et al. Descriptive epidemiology of febrile respiratory illness in a Mexican population.
- ◆ Wu J, et al. Multiplex polymerase chain reaction assay for detection of adenovirus in patient specimens.
- ◆ Barrozo C, et al. Molecular characteristics of methicillin-resistant *Staphylococcus aureus* isolates from community outbreaks in San Diego, California, 2002.

The Sixth Annual Force Health Protection Conference, August 11-17, 2003, Albuquerque, NM.

- ◆ Hawksworth A, et al. An alternative method for classification of febrile respiratory illness rates at U.S. military basic training centers.
- ◆ Strickler J, et al. Antibiotic resistance and emm-gene typing of clinical *Streptococcus pyogenes* isolates from U.S. military recruits.
- ◆ Gunnill R, et al. Pneumonia etiology compared to self-reported symptoms: data from a double-blind placebo-controlled pneumococcal vaccine trial in military trainees.
- ◆ Freed N, et al. Application of LightCycler® for the detection of influenza viruses and adenoviruses by fluorogenic RT PCR and PCR onboard a U.S. Naval aircraft carrier.

The Forty-Third Navy Occupational Health and Preventive Medicine Workshop, 18-26 March 2004, Chesapeake, VA.

- ◆ Barrozo C, et al. An outbreak of conjunctivitis at a Marine Corps recruit training site.
- ◆ Dejesa L, et al. Respiratory pathogen detection among the U.S. naval fleet.
- ◆ Fuller J, et al. Deployment surveillance for SARS: Cobra Gold 03.
- ◆ Gratwick K, et al. Adenovirus-associated deaths within the Department of Defense: winter 2003/2004.
- ◆ Osuna M, et al. Shipboard fluorogenic PCR for rapid detection of respiratory pathogens.
- ◆ Russell K, et al. Influenza vaccine effectiveness among U.S. military basic trainees, 2003-04.

- ◆ Strickler J, et al. Laboratory-based surveillance studies at the DoD Center for Deployment Health Research, Respiratory Disease Laboratory, Naval Health Research Center.

Recent Publications

- ◆ Barrozo CP, Russell KL, Smith TC, Hawksworth AW, Ryan MA, Gray GC. National Department of Defense surveillance data for antibiotic resistance and emm gene types of clinical group A streptococcal isolates from eight basic training military sites. *J Clin Microbiol.* 2003 Oct;41(10):4808-11.
- ◆ Blasiole DA, Metzgar D, Daum LT, Ryan MAK, Wu J, Wills C, Le C, Freed N, Hansen CJ, Gray GC, Russell KL. Molecular analysis of adenovirus isolates from previously vaccinated young adults. *J Clin Microbiol*, in press.

Collaborations and Grants in Consideration

- ◆ Molecular analyses of recent influenza isolates, in collaboration with Dr. Jeffrey Taubenberger, Armed Forces Institute of Pathology.
- ◆ Viral isolation and molecular evaluation of coronavirus 229E, in collaboration with Dr. Kathryn Holmes, University of Colorado.

Please contact the newsletter staff with any questions, comments, or suggestions regarding the information in this issue.

Jennifer Strickler
Tel: (619) 553-8163

DSN: 553-8163

Fax: (619) 553-7601

striker@nhrc.navy.mil

Tony Hawksworth
Tel: (619) 553-7607

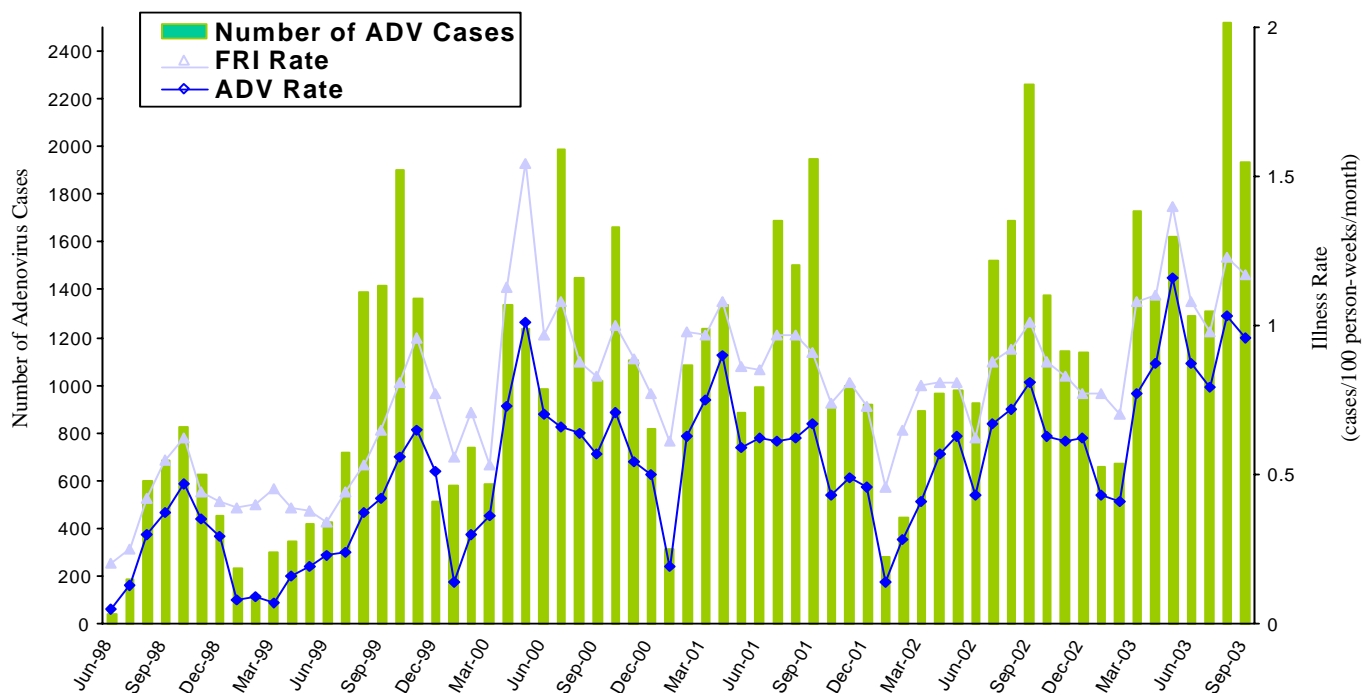
DSN: 553-7607

Fax: (619) 553-7601

hawksworth@nhrc.navy.mil

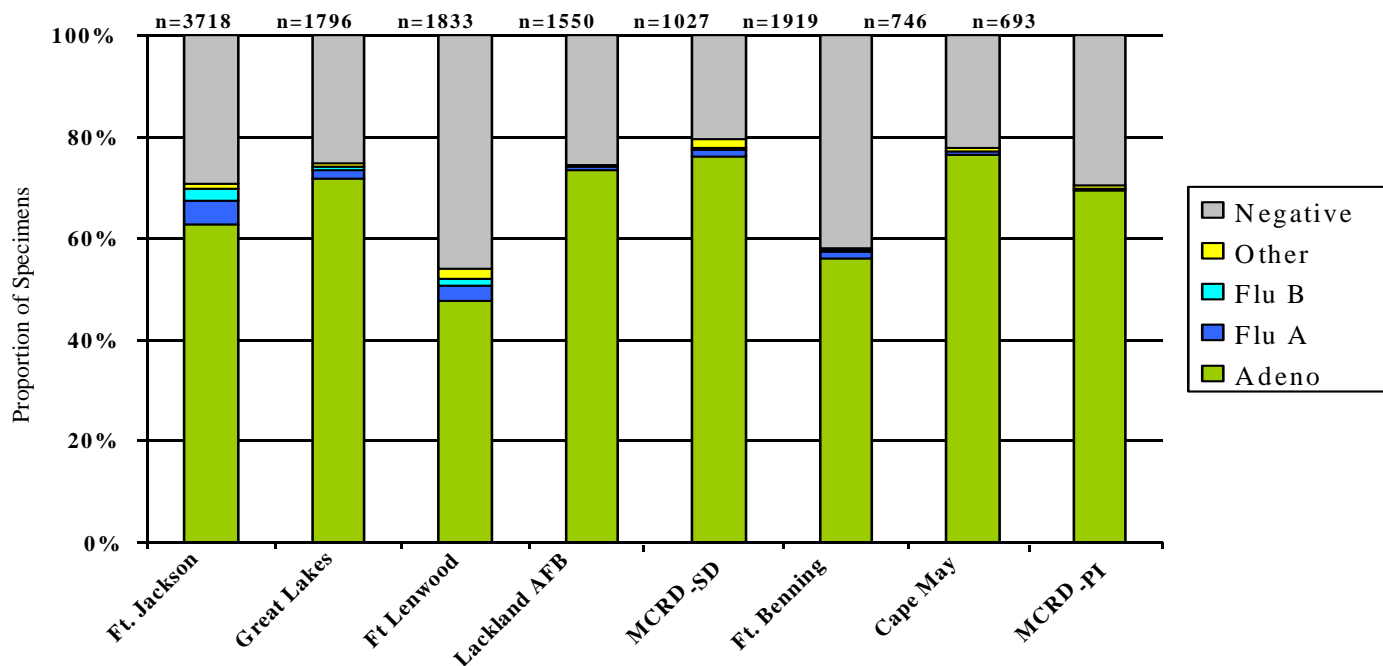
We would like to thank the staffs at our collaborating institutions for their hard work and dedication!

Combined Febrile Respiratory Illness (FRI) and Adenovirus (ADV) Morbidity Among Symptomatic Trainees at Eight Military Training Centers

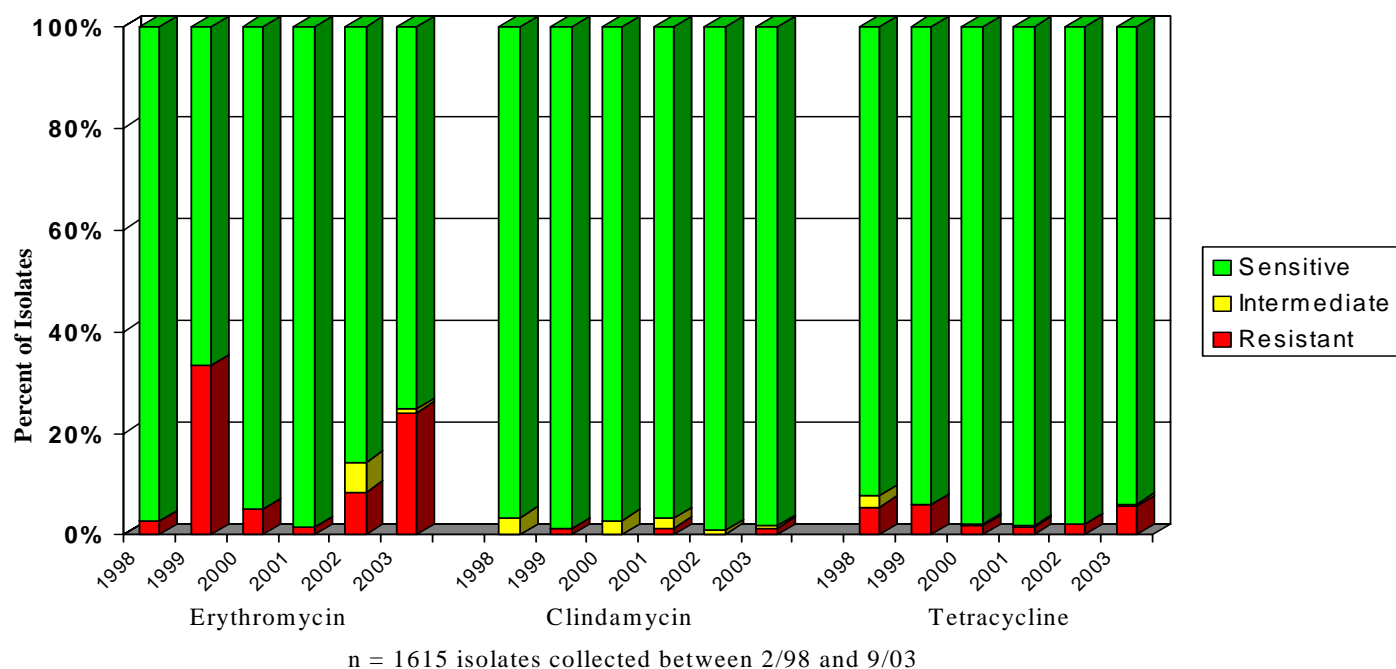


Distribution of Viral Test Results by Site

June 1998 - September 2003
n=13,282



Antibiotic Resistance Patterns of Clinical *Streptococcus pyogenes* Isolates Over Time



Antibiotic Resistance Patterns of Clinical *Streptococcus pneumoniae* Isolates Over Time

